Acute Respiratory Distress Syndrome (ARDS) Phenotyping

Running title: ARDS sub-phenotypes

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Clinically, the Berlin ARDS definition describes acute respiratory distress syndrome (ARDS) as acute hypoxemic respiratory failure, that is not fully explained by cardiac failure or fluid overload, that develops within seven days of clinical recognition of a known risk factor, with bilateral radiographic opacities that are not fully explained by effusions, lobar/lung collapse, or nodules. Three risk strata were defined, based on the severity of hypoxemia represented by the ratio of partial pressure of oxygen in arterial blood to inspired oxygen concentration (PaO_2/FiO_2 ratio), assessed at a minimum positive end-expiratory pressure (PEEP) of 5 centimetres of water [1]. Hospital mortality worsens with severity of hypoxaemia and thus grade of ARDS (from 35% in mild ARDS to 46% in severe ARDS) [1, 2].

The ARDS consensus definitions to-date have mainly relied on feasible clinical criteria, which helps to group patients together for inclusion in clinical trials and for clinical management [1, 3]. This generates clinical and biological differences in observable patient characteristics. These differences are associated with inter-individual variation in the risk of illness, risk of outcomes from the illness, response to treatments administered for the illness and combinations thereof (referred to as heterogeneity)[4, 5]. For example, pneumonia is a common risk factor for ARDS [2, 3, 6]. However, not every patient admitted with pneumonia develops ARDS. One possible explanation is that there are differences in host responses between patients, as observed by variation in biomarker profiles for the same risk factor [7] . Amongst patients with pneumonia who develop ARDS, the severity and outcome vary between cohorts. This ARDS heterogeneity has contributed towards many statistically negative randomised controlled trials (RCT) [6].

Thus, identifying subgroups of ARDS patients (referred to as ARDS sub-phenotypes in the literature) who either have a higher risk of mortality (prognostic enrichment), or differences in treatment responses and/or similar biological mechanisms that are modifiable (predictive enrichment) could enable stratified and/or precision medicine [5]. To-date, two ARDS sub-phenotypes have been reported from reanalyses of five RCTs [8-12], and in one observational cohort study [13], where the sub-phenotype membership was determined by similarities in the observable clinical and biological features. Although, all six studies report two ARDS sub-phenotypes, they differ in the biomarkers, clinical characteristics, and analytic approaches used. All RCT reanalyses used biomarker and clinical

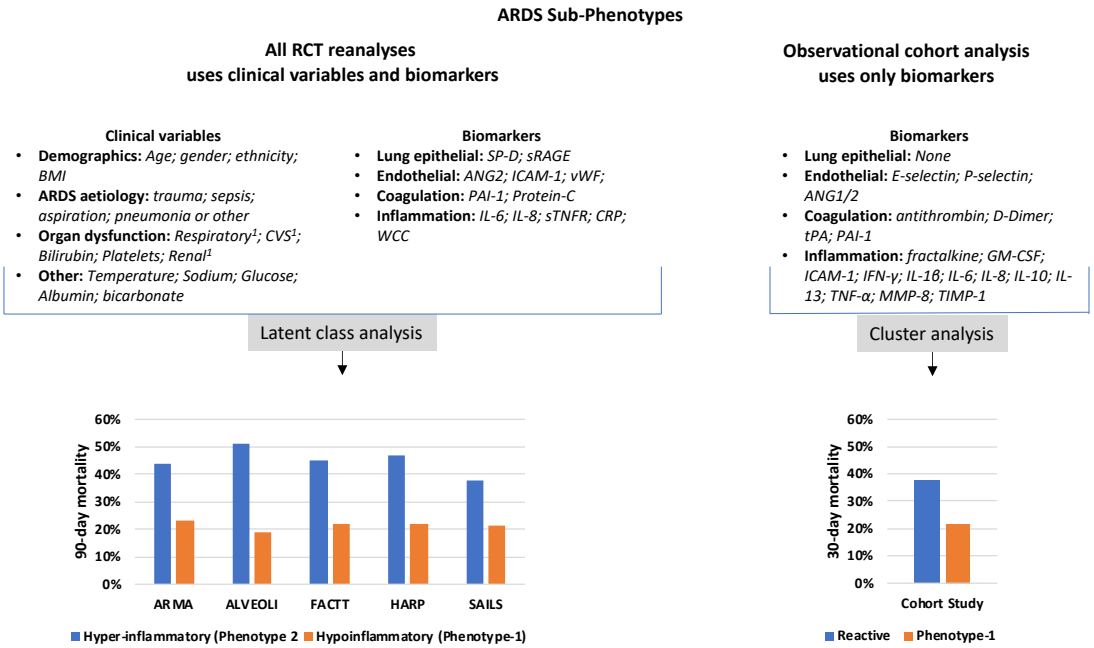
characteristics with latent class analyses to determine ARDS sub-phenotypes [8-12]. All RCTs report a *hyperinflammatory* sub-phenotype characterised by higher plasma concentrations of inflammatory biomarkers, greater vasopressor use, lower serum bicarbonate concentrations, and a higher prevalence of sepsis, when compared to the more common hypoinflammatory (Phenotype-1) sub-phenotype [8-12]. This *hyperinflammatory* sub-phenotype has higher mortality (prognostic enrichment) and different treatment responses to PEEP, simvastatin, and fluid management (predictive enrichment) [8-11], which may reflect true biological effect modification. Importantly, these two phenotypes could be distinguished with a three-variable model of serum interleukin-8 (IL-8) or IL-6, bicarbonate, and soluble tumor necrosis factor receptor-1 (sTNFR-1), in patients enrolled within the ARDS Network RCTs of lower versus higher tidal volume ventilation (ARMA), higher versus lower positive end-expiratory pressure (ALVEOLI), and the Fluid and Catheter Treatment Trial (FACTT) [8, 9]. In contrast, the observational cohort study used only biomarker data with clustering analysis to determine ARDS sub-phenotypes [13]. The *reactive* sub-phenotype reported in the observational study had higher mortality when compared to the *uninflamed* sub-phenotype [13] and could be distinguished with a four-variable biomarker panel consisting of IL-6, interferon gamma, angiopoietin 1, angiopoietin 2, and plasminogen activator inhibitor-1 [Figure-1]. While up to a third of patients with ARDS progressed to more severe risk strata over the first 7 days [1, 2], these ARDS sub-phenotypes remained stable over first three days of enrolment into ARMA and ALVEOLI trials [11].

Prior to any attempts at incorporating management directed to ARDS sub-phenotypes into routine clinical practice, a number of important questions need to be answered. The prevalence of reactive sub-phenotypes was twice as common in the observational cohort study compared to the *hyperinflammatory* sub-phenotypes in RCTs. It is not clear if this is due to selection bias inherent to randomized trials or to differences in biology. We need to ascertain whether the *reactive and hyperinflammatory* sub-phenotypes are phenotypically and/or biologically similar. As infection is the most common risk factor for ARDS, similarities of ARDS sub-phenotypes to sepsis sub-phenotypes should be explored. Prospective validation studies with a standardised approach to ARDS sub-phenotyping are needed and could be an international inception cohort study similar to the LUNG-SAFE study [2]. As radiology and need for minimum PEEP are challenging elements of the Berlin Definition, acute hypoxemic respiratory failure (i.e., $PaO_2/FiO_2 \leq 300$ regardless of PEEP level) could

be the inception cohort, which then could be used to identify ARDS patients within the AHRF cohort. The study aims are then to determine similarities and differences between acute hypoxemic respiratory failure and ARDS, alongside validating ARDS sub-phenotypes. Studying biological signals in this context requires a comprehensive set of data of different biological dimensions (e.g., gene expression on leukocytes [leukocyte transcriptome], and altered patterns of protein components in blood [proteome], in addition to detailed clinical phenotyping). This research should resemble a system biology research cycle and could begin with the hypothesis that there are at least two biologically distinct ARDS sub-phenotypes and that these ARDS sub-phenotypes have differences in risk of death and treatment response. There is a clinical need to identify biomarkers that are associated with treatment response, independent of group membership. Therefore, consensus on biological sampling time-points, biological dimension measured, and a standard minimum set of consensus biomarkers is required. The biological sampling should aim to reflect the insults to the alveolar capillary membrane (ACM) (exudative phase), deposition of provisional matrix with proliferation of airway progenitor cells (proliferative phase), or interstitial and intra alveolar fibrosis (fibrotic phase) of ARDS. This minimum set of markers could then be used to delineate discriminant markers of ARDS sub-phenotypes that provide prognostic enrichment with either a greater probability of therapeutically valid similarities or a greater likelihood of treatment response.

Legend

Figure-1: ARDS Phenotyping



	ARMA N=473	ALVEOLI N=549	FACTT N=1000	HARP N=539	SAILS N=745
% Hyper-inflammatory	32.8%	26.5%	27.2%	34.5%	37.2%
Phenotype discriminant markers	sTNFR-1; IL-6; vasopressor use	sTNFR-1; IL-6; vasopressor use	sTNFR-1; IL-8; bicarbonate	sTNFR-1; IL-6; low platelets; vasopressor	sTNFR-1; IL-8; bicarbonate

	Cohort Study N=700
% Reactive phenotype	51.9%
Phenotype discriminant markers	IL-6; IFN-γ; ANG1/2; PAI-1

Broadly, the term phenotype refers to observable characteristics of an organism. The observable differences in clinical and biological characteristics could be used to group ARDS patients, referred to as ARDS sub-phenotypes. ARDS sub-phenotypes have been reported from five randomised controlled trials and one observational cohort study. There are differences in the variables and the method used to define ARDS sub-phenotypes, which are summarised in the figure.
[1]The Respiratory system variables included minute ventilation, mean airway pressure, plateau pressure, respiratory rate, tidal volume, positive end-expiratory pressure; partial pressure PaO_2 of carbon dioxide ($PaCO_2$) and ratio of partial pressure of oxygen in arterial blood to inspired oxygen concentration (PaO_2/FiO_2 ratio). The Cardiovascular system variables include highest heart rate, lowest systolic blood pressure and vasopressor use. The renal variables included creatinine, urea and urine output. ANG1/2, angiopoietin 1 and 2; BMI, body mass index; CRP – C reactive protein; GM-CSF, granulocyte-monocyte colony stimulating factor; HCO3, bicarbonate; ICAM-1, intracellular adhesion molecule-1; IFN-γ, interferon gamma; IL, interleukins 6, 8, 10, 13; IL-1β, interleukin-1 beta; LDH – lactate dehydrogenase; MMP-8, matrix metalloproteinase-8; P/F, PaO_2/FiO_2 ratio; PAI-1, plasminogen activator inhibitor-1; PCT – procalcitonin; sRAGE, soluble receptor for advanced glycation end products; RCT, randomised controlled trial; SP-D, surfactant protein-D; sTNFR-1, soluble tumour necrosis factor receptor-1; TIMP-1, tissue inhibitor of metalloproteinase-1; TNF-α, tumor necrosis factor-alpha; TNFR – tumor necrosis factor receptor; tPA, tissue plasminogen activator; vWF, von-Williebrand's factor; VeGF – vascular endothelial growth factor; WBC, white blood cell count.

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